

**Medicament for inhibiting the expression of a given gene**

The invention relates to a medicament and to a use of  
5 double-stranded oligoribonucleotides.

DE 196 31 919 C2 describes an antisense RNA with specific secondary structures, the antisense RNA being present in the form of a vector encoding it. The  
10 antisense RNA takes the form of an RNA molecule which is complementary to regions of the mRNA. Binding to these regions brings about an inhibition of gene expression. This inhibition can be employed in particular for the diagnosis and/or therapy of  
15 diseases, for example tumour diseases or viral infections. The disadvantage is that the antisense RNA must be introduced into the cell in an amount which is at least as large as the amount of the mRNA. The efficacy of the known antisense methods is not  
20 particularly high.

US 5,712,257 discloses a medicament which contains mispaired double-stranded RNA (dsRNA) and bioactive mispaired fragments of dsRNA in the form of a ternary  
25 complex with a surfactant. The dsRNA used in this context consists of synthetically generated nucleic acid simplexes without a defined base sequence. The simplexes undergo random base pairing with each other, giving rise to mispaired duplexes. The known dsRNA  
30 serves for inhibiting the replication of retroviruses such as HIV. The retroviral genome consists of double-stranded RNA which, during replication of the retrovirus, binds a variety of proteins. Binding of these proteins, and thus replication of the virus, can  
35 be inhibited if unspecific dsRNA is introduced into the infected cells in high concentrations. The result is competition between the unspecific dsRNA and the double-stranded viral RNA. The inhibitory effect, or the efficacy, of this method is poor.

Fire, A. et al., NATURE, Vol. 391, pp. 806 describes that dsRNA one strand of which has segments which are complementary to a nematode gene to be inhibited will  
5 inhibit the expression of this gene with high efficacy. It is believed that the particular efficacy of the dsRNA used in cells of the nematode is not based on the antisense principle, but, possibly, on catalytic properties of the dsRNA. Nothing is mentioned in this  
10 paper on the efficacy of specific dsRNA with regard to the inhibition of gene expression, in particular in human cells.

It is an object of the present invention to do away  
15 with the disadvantages of the prior art. In particular, it is intended to provide as efficacious a medicament as possible, or as efficacious a use as possible for the preparation of a medicament, with the aid of which an inhibition of the expression of a given gene can be  
20 brought about.

This object is achieved by the features of Claims 1, 2, 13 and 14. Advantageous embodiments can be seen from Claims 3 to 12 and 15 to 26.  
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In accordance with the invention, there is provided a medicament with at least one double-stranded oligoribonucleotide (dsRNA) for inhibiting the expression of a given gene, where one strand of the dsRNA has at least  
30 segments which are complementary to this gene. Surprisingly, it has emerged that dsRNA is suitable as medicament for inhibiting the expression of a given gene in human cells. The inhibition is brought about even at concentrations which are at least one order of  
35 magnitude lower than when single-stranded oligoribonucleotides are used. The medicament according to the invention is highly efficacious. Fewer side-effects are to be expected.

In accordance with the invention, there is furthermore provided a medicament with at least one vector for encoding double-stranded oligoribonucleotides (dsRNA) for inhibiting the expression of a given gene, where  
5 one strand of the dsRNA has at least segments which are complementary to this gene. The proposed medicament has the abovementioned advantages. By using a vector, savings, in particular with regard to the production, can be made.

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In the event that dsRNA is used as an active substance, it has proved advantageous for the dsRNA to be packaged in micellar structures, preferably in liposomes. Likewise, the dsRNA can be enclosed in viral natural  
15 capsids or in synthetic capsids prepared chemically or enzymatically, or structures derived therefrom. The abovementioned features allow the dsRNA to be introduced into given target cells.

20 In a further embodiment, the dsRNA has 10 to 1 000, preferably 250 to 350, base pairs. Such a dsRNA, or a vector provided for encoding the same, can be generated synthetically or enzymatically using customary methods.

25 The gene to be inhibited, preferably an oncogene, can be capable of expression in eukaryotic cells or in pathogenic organisms, preferably in plasmodia. It may be part of a virus or viroid, preferably a virus or viroid which is pathogenic to humans. The proposed  
30 medicament permits the therapy of genetically controlled diseases, for example cancer and viral diseases.

The virus or viroid may also take the form of a virus  
35 or viroid which is pathogenic to animals or plants. In this case, the medicament according to the invention also permits the treatment of animal or plant diseases.

According to a further embodiment, segments of the dsRNA are in double-stranded form. To prevent degradation in the cell, their termini may be modified. This makes enzymatic attacks more difficult.

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In accordance with a further aspect of the invention, there is provided a use of double-stranded oligoribonucleotides for the preparation of a medicament for inhibiting the expression of a given gene, where one strand of the dsRNA has at least segments which are complementary to this gene. Surprisingly, dsRNA is suitable for the preparation of a medicament for inhibiting the expression of a given gene. When dsRNA is used, inhibition is brought about even at concentrations which are one order of magnitude lower than when single-stranded oligoribonucleotides are used. The use according to the invention thus makes possible the preparation of particularly efficacious medicaments.

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In accordance with a further aspect of the invention, there is provided the use of a vector for encoding double-stranded oligoribonucleotides (dsRNA) for the preparation of a medicament for inhibiting the expression of a given gene, where one strand of the dsRNA has at least segments which are complementary to this gene. The use of a vector makes possible the preparation of particularly inexpensive and efficacious medicaments.

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As regards the embodiments intended for use, reference is made to the description of the above features.

Use example:

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An RNA simplex, which can be seen from the single sequence listing, has been synthesized enzymatically with the aid of conventional methods.

The RNA simplex which is complementary thereto has also been synthesized. Then, the simplex and the complementary simplex were combined to give the dsRNA. The resulting dsRNA contains a segment of the  
5 cytomegalovirus immediate early gene.

Test protocol:

A plasmid vector, the use of which allowed the required dsRNA to be prepared, was constructed. To construct  
10 this T7/SP6 transcription plasmid, a polymerase chain reaction (PCR) for amplifying the 363 base pairs starting at the 5' end of the cytomegalovirus immediate early gene was carried out. The template used was commercially available cytomegalovirus positive control  
15 DNA from the HeLaScribe<sup>®</sup> Nuclear Extract *in-vitro* transcription kit from Promega. The primers used were oligodeoxyribonucleotides, whose sequences were identical with, or complementary to, the termini of the above-stated region of the immediate early gene. The  
20 cloning vector which was used for the resulting PCR product was the vector pGEM<sup>®</sup>-T (Promega). *E. coli* XL1-blue was transformed. Plasmid DNA of a selected clone whose sequence had been verified by partial sequencing was linearized with *Nco*I or *Sal*I and used as template  
25 for an *in-vitro* transcription with SP6 or T7 RNA polymerase (RiboMAX<sup>™</sup> *in-vitro* transcription kit, Promega).

The resulting oligoribonucleotides were purified and  
30 combined in equimolar amounts in sodium phosphate buffer (pH 6.5) in the presence of 100 mM NaCl. After the mixture had been heated briefly at 95°C, it was cooled slowly over the course of approx. 2.5 hours, whereby the formation of dsRNA by pairing of the two  
35 complementary simplexes was brought about.

Assay system with human nuclear extract:

Using the HeLaScribe<sup>®</sup> Nuclear Extract *in-vitro* transcription kit from Promega, the transcription

efficiency of the above-stated region of the cytomegalovirus immediate early gene in the presence of the two single-stranded oligoribonucleotides and of the dsRNA was determined. This was done with the aid of the radioactivity, incorporated into the run-off transcripts of the [ $\alpha^{32}\text{P}$ ] ATP used as substrate. Separation of the free ATP from the transcript generated was carried out by gel electrophoresis. The gel was evaluated with the aid of a radioactivity detector (Instant-Imager).

Result and conclusion:

It emerged that the amount of transcript in the presence of dsRNA was markedly reduced in comparison with the control set-up without RNA and also with the set-ups with single-stranded RNA. The efficacy of the dsRNA was achieved even when only small amounts, i.e. less than 10% of the RNA concentration required in antisense technology for inhibiting translation, were added. The inhibitory effect of single-stranded antisense RNA would not be detectable in this assay system since this would detect inhibition at the translational level, while transcription was studied in the present case. The reduction in the transcript quantity of a gene in the presence of dsRNA, which was observed for the first time in humans in the present context, clearly shows inhibition of expression of the gene in question. This effect can be attributed to a novel mechanism brought about by the dsRNA.

Patent Claims

1. Medicament with at least one double-stranded oligoribonucleotide (dsRNA) for inhibiting the expression of a given gene, where one strand of the dsRNA has at least segments which are complementary to this gene.
2. Medicament with at least one vector for encoding double-stranded oligoribonucleotides (dsRNA) for inhibiting the expression of a given gene, where one strand of the dsRNA has at least segments which are complementary to this gene.
3. Medicament according to Claim 1, where the dsRNA is packaged in micellar structures, preferably in liposomes.
4. Medicament according to Claim 1, where the dsRNA is enclosed in viral natural capsids or in synthetic capsids prepared chemically or enzymatically, or structures derived therefrom.
5. Medicament according to one of the preceding claims, where dsRNA has 10 to 1 000, preferably 250 to 350, base pairs.
6. Medicament according to one of the preceding claims, where the gene to be inhibited, preferably an oncogene, is capable of expression in eukaryotic cells.
7. Medicament according to one of the preceding claims, where the gene to be inhibited is capable of expression in pathogenic organisms, preferably in plasmodia.
8. Medicament according to any of the preceding claims, where the gene to be inhibited is part of

a virus or viroid.

- 5 9. Medicament according to Claim 8, where the virus is a virus or viroid which is pathogenic to humans.
- 10 10. Medicament according to Claim 8, where the virus or viroid is a virus or viroid which is pathogenic to animals or plants.
11. Medicament according to one of the preceding claims, where segments of the dsRNA are in double-stranded form.
- 15 12. Medicament according to one of the preceding claims, where the termini of the dsRNA are modified to prevent degradation in the cell.
- 20 13. Use of double-stranded oligoribonucleotides (dsRNA) for the preparation of a medicament for inhibiting the expression of a given gene, where one strand of the dsRNA has at least segments which are complementary to this gene.
- 25 14. Use of a vector for encoding double-stranded oligoribonucleotides (dsRNA) for the preparation of a medicament for inhibiting the expression of a given gene, where one strand of the dsRNA has at least segments which are complementary to this gene.
- 30 15. Use according to Claim 13, where the dsRNA is packaged in micellar structures, preferably in liposomes.
- 35 16. Use according to Claim 13, where the dsRNA is enclosed in viral natural capsids or in synthetic capsids prepared chemically or enzymatically, or structures derived therefrom.

17. Use according to one of Claims 13 to 16, where dsRNA has 10 to 1 000, preferably 250 to 350, base pairs.
- 5 18. Use according to one of Claims 13 to 17, where the gene to be inhibited, preferably an oncogene, is expressed in eukaryotic cells.
- 10 19. Use according to one of Claims 13 to 18, where the gene to be inhibited is expressed in pathogenic organisms, preferably in plasmodia.
- 15 20. Use according to one of Claims 13 to 19, where the gene to be inhibited is part of a virus or viroid.
21. Use according to Claim 20, where the virus is a virus or viroid which is pathogenic to humans.
- 20 22. Use according to Claim 20, where the virus or viroid is a virus or viroid which is pathogenic to animals or plants.
- 25 23. Use according to one of Claims 13 to 22, where segments of the dsRNA are in double-stranded form.
24. Use according to one of Claims 13 to 23, where the termini of the dsRNA are modified to prevent degradation in the cell.
- 30 25. Use according to one of Claims 13 to 24, where the medicament is capable of being injected into the bloodstream or the interstitium of the organism which is to undergo therapy.
- 35 26. Use according to one of Claims 13 to 25, where the dsRNA, or the vector encoding it, is incorporated in bacteria or microorganisms.

## Abstract

The invention relates to a medicament with at least one double-stranded oligoribonucleotide (dsRNA) for inhibiting the expression of a given gene, where a strand of the dsRNA has at least segments which are complementary to this gene.

# SEQUENCE LISTING

<110> Kreutzer, Roland  
Limmer, Stefan

<120> Medicament for inhibiting the expression of a  
given gene

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